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Immunotherapy of rheumatic diseases – practice and prospects

Gabrielle Kingsley, Gabriel Panayi and Jerry Lanchbury

Present treatments for rheumatic diseases are both toxic to patients and largely ineffective in modifying the disease process. This report, based on a meeting recently held in London, investigates how far recent molecular and immunological advances can be converted into more effective, less toxic and, above all, more specific therapies.*

The greatest obstacle to developing specific effective therapy for rheumatoid arthritis (RA) has been lack of understanding of aetiology. Nevertheless, based on present knowledge, a pathogenetic model can be constructed (Fig. 1) in which rheumatoid antigen(s) is presented in the context of a limited range of major histocompatibility complex (MHC) class II structures to the disease-promoting $CD4^+$ T cells that become activated[1,2]. These, in turn, activate other cells including macrophages, B cells, other T cells and synoviocytes to release effector molecules such as cytokines, growth factors, antibodies and degradative enzymes. This leads to synovial inflammation and proliferation which cause joint destruction. It is likely that this immune activation will trigger a variety of immunoregulatory mechanisms but these are as yet ill-defined. Whether the critical MHC–antigenic peptide–T-cell receptor interactions occur in central lymphoid organs or whether this activation occurs primarily *in situ* after migration of T cells to the synovium is not known. In either case migration of T cells must play an important role in generating synovitis. Each component of this pathogenetic pyramid is a potential target for immunotherapeutic intervention.

Therapy directed against cytokines

There is a consensus that the monokines interleukin 1 (IL-1), IL-6, granulocyte–macrophage colony-stimulating factor (GM-CSF) and tumour necrosis factor α (TNF-α) are present within the joint in rheumatoid arthritis patients at high concentrations but there is considerable controversy about T-cell-derived cytokines such as IL-2, IL-4 and gamma-interferon (IFN-γ)[1,3]. The role of IL-7, IL-8, IL-9 and IL-10 is even more uncertain although, interestingly, IL-8 has recently[4] been shown to be elevated in RA synovial fluid compared with other inflammatory arthritides.

Several cytokine-based therapeutic approaches are being used experimentally but, as yet, none has been applied to humans. Strategies include anti-cytokine monoclonal antibodies, soluble cytokine receptor proteins and specific cytokine inhibitors, notably the naturally-occurring IL-1 receptor antagonist (IL-1ra)[5]. One potential practical problem in the therapeutic use of these small molecular weight proteins is the inordinately high doses required; for example, in endotoxic shock in rabbits a total of 100 mg kg^{-1} IL-1ra was required to achieve a good therapeutic effect[6] However, the most important question regarding cytokine intervention in rheumatic disease lies not in its technical feasibility but in the likely effect of interfering with only one cytokine within what is undoubtedly a very complex network. It seems highly improbable that a single cytokine holds the key to RA synovitis.

What of other targets for therapy? Macrophages are possible targets[3] but have not yet been investigated. Autoantibodies, notably rheumatoid factor, were at one time popular

*The First International Symposium on the Immunotherapy of the Rheumatic Diseases was held in London, UK on 20–22 February 1991 and was organized by Gabrielle Kingsley, Gabriel Panayi and Jerry Lanchbury.

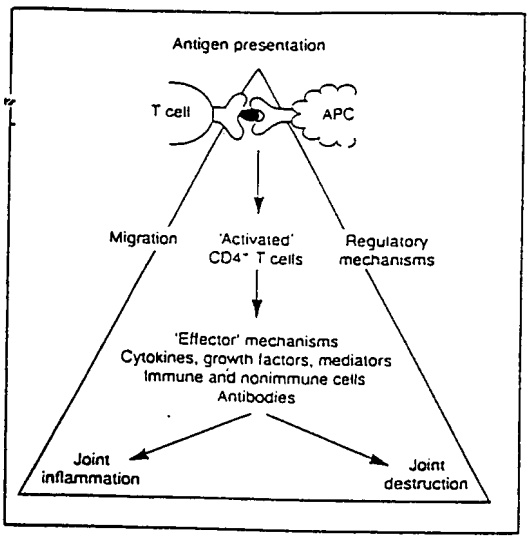

Fig. 1. *A model for the pathogenesis of rheumatoid arthritis.*

targets for therapy but the lack of effect of plasmapheresis on RA synovitis provides fairly conclusive evidence against them. T-cell therapy is currently favoured.

Therapy directed towards T cells

There is overwhelming evidence that supports a central position for the T cell in rheumatoid synovitis: immunohistological studies of RA synovium demonstrate perivascular aggregates of activated $CD4^+$ T cells closely applied to antigen-presenting cells[7]; T cells specific for the inducing agent from animals with experimental arthritides, such as adjuvant arthritis and streptococcal cell wall arthritis, can transfer disease; in human inflammatory arthritides of known cause (reactive arthritis) T cells specific to the inciting antigen are found in the joint. The critical role of T cells in RA is corroborated by the striking association of particular HLA class II alleles with the disease, given that these molecules restrict the presentation of antigenic peptides to $CD4^+$ T cells[8]. Most convincingly, early attempts at T-cell-directed immunotherapy in humans, such as thoracic duct drainage, total lymphoid irradiation and lymphocytapheresis, while neither practical nor safe as routine therapy, resulted in disease remission. The recent beneficial use of cyclosporin A, which, primarily, interferes with T-cell function, in RA further supports their importance.

Several studies of monoclonal antibodies directed against T-cell targets have been undertaken in RA patients. A monoclonal antibody (RFT2) against the T-cell activation antigen CD7 (Ref. 9) (shown to be effective in transplantation) had little clinical benefit in RA, although $CD7^+$ T cells disappeared both from blood and synovium. A better clinical result was obtained using Campath-6, a monoclonal antibody against the IL-2 receptor: two out of three patients improved for at least three months[10]. However, the most exciting results in RA come from the, by now extensive, studies using anti-CD4 antibody. In Europe, about 100 patients have been treated with murine and, more recently, chimeric human–mouse monoclonal anti-CD4 antibodies in open studies[11,12]. Approximately 60% of the patients show a significant clinical improvement, similar to that seen with methotrexate or cyclosporin A, and lasting on average from two to three months. No serious toxicity has been observed and only low titre anti-mouse antibodies develop; thus retreatment is possible. Double-blind placebo-controlled multicentre studies are now underway.

Disappointingly, the mode of action of anti-CD4 antibodies remains unclear. An anti-idiotypic response does not appear to develop and there is no long-term immunosuppression: in most patients, only a transient fall in $CD4^+$ cells is seen. Is this evidence for reprogramming of the immune system as proposed by Waldmann[13]?

Another agent used in treatment is anti-CD5 antibody coupled to ricin (although it is not established that ricin is necessary for its action). Promising results have been achieved, especially in early disease, but controlled studies are awaited[14]. The process of T-cell migration should also be considered as a target. No studies in RA have yet been conducted but anti-lymphocyte function-associated molecule 1 (LFA-1) antibody has been used in human bone marrow transplantation.

Although the anti-CD4 antibody looks a promising treatment, it is a nonspecific mode of immunointervention directed against all CD4 cells. Of greater interest is the possibility of therapy directed against the apex of the pyramid (Fig. 1), that is the specific T cells, antigenic peptides and MHC molecules involved in RA.

T-cell vaccination

In T-cell vaccination, first developed in animal models by Irun Cohen[15], subpathogenic doses of disease-promoting T cells are injected into the skin, inducing regulatory T cells that downregulate activity. For example, a T-cell clone, A2b, derived from an animal with adjuvant arthritis, could transfer disease to naive animals. This A2b clone could protect against arthritis and suppress established disease if it was both activated and chemically fixed or irradiated. Vaccinated animals develop a cell-mediated immune response against the A2b T-cell clone. Interestingly, in some animal models the anti-vaccine response appears to amplify a pre-existing immunoregulatory T-cell response.

Since the eliciting antigen in most rheumatic diseases is unknown, the production of specific T-cell lines or clones to be used as vaccines is clearly impossible. However, in adjuvant arthritis it is possible to expand the regulatory T cells by antigen-nonspecific stimulation of T cells from central lymphoid organs[15]. Based on this work, activated T cells have been expanded from the joints of patients with RA and have been used as vaccines. These trials are underway in Leiden, Mainz and London. The initial concern of all investigators has been to establish the safety of the procedure. Approximately 15 patients with RA have been treated to date without any significant side effects but it is too early to consider clinical effectiveness.

The T-cell repertoire and immunization with T-cell receptor peptides

By its very nature, the technique of T-cell vaccination requires a preparation unique to each patient. If the relevant antigens on the T cell could be identified then a standard vaccine could be developed. One leading candidate for the stimulating moiety is the T-cell receptor (TCR). For example, in experimental allergic encephalomyelitis (EAE), disease-inducing T cells express a limited repertoire of TCRs, predominantly $V_\alpha 2V_\beta 8$; in the Lewis rat. A 21

amino acid peptide, including the complementarity-determining region (CDR) 2 of $V_\beta 8$, was effective in protecting against and treating rats with EAE[16]. Vandenbark and colleagues suggest that these effects are due to the augmentation of a pre-existing immunoregulatory network. Confirmation of these exciting findings is eagerly awaited.

The major problem in the application of this technique to the treatment of RA is that it requires that the disease is caused by T cells with a restricted TCR usage. Conflicting evidence exists for oligoclonal αβ TCR use in RA. This may reflect the diversity of material studied, which includes uncultured and *in vitro* IL-2 expanded synovial fluid and membrane T cells, examined mainly by Southern blotting and C_β probe hybridization (M. Steinmetz and Y. Uematsu, unpublished). Recently anchor and inverse polymerase chain reaction (PCR), coupled with sequencing and V and J region oligonucleotide screening of αβ TCR libraries, have been applied to joint and peripheral blood T cells. This technique provides a sensitive and unselective estimate of the T-cell repertoire and has been exploited[17] to analyse peripheral blood, synovial fluid and synovial membrane T cells in HLA-DR4$^+$ RA patients. Initial analysis shows a skewing towards $V_\beta 2.1$ use in synovial T cells. In one patient the $V_\beta 2.1$ segment was preferentially associated with $J_\beta 2.3$, which may indicate clonal expansion. It is not clear whether such extreme and site-directed distortion of repertoire relates to chronic ongoing joint disease or to short-term infection, since it is known that $V_\beta 2.1$ populations are expanded by bacterial superantigens.

MHC-binding peptides

Genetic and epidemiological studies suggest that the HLA component of susceptibility to RA is a relatively conserved pentapeptide sequence that locates to the putative α helical region bordering the peptide-binding groove of RA-associated HLA-DR alleles[8]. Recent studies have reinforced the view that this epitope is preferentially associated with chronic RA (particularly disease involving extra-articular manifestations) and is not equally prevalent in all populations. Modelling the contact sites in susceptibility versus nonsusceptibility alleles over this helical region suggests that variability of peptide contact sites may be critical in the disease process.

The binding of specific peptide by MHC molecules represents an attractive target for therapeutic disruption by peptide blockade providing that soluble MHC blockers can be exchanged for pre-bound peptide *in vivo* without inducing novel immunogenicity. These criteria have been satisfied in model experimental systems (reviewed in Ref. 18) but, in animal models of autoimmune disease, MHC-blocking peptides have been successfully used only for prophylaxis and not for treatment.

In experimental models, controversy exists about whether selective blocking peptides should be sequence modifications of the original disease-inducing peptide, or high-affinity peptides that are unrelated in sequence. The *in vivo* effects of the former are difficult to interpret since they may cause deviation rather than blockage of the immune response. In terms of a therapy for RA, it is likely that screening of randomly-derived peptides for binding to suitable HLA-DR molecules represents a reasonable first step since convincing candidate peptides for sequence modification have yet to be described.

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TREATMENT OF RHEUMATOID ARTHRITIS WITH CHIMERIC MONOCLONAL ANTIBODIES TO TUMOR NECROSIS FACTOR α

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Objective. To evaluate the safety and efficacy of a chimeric monoclonal antibody to tumor necrosis factor α (TNFα) in the treatment of patients with rheumatoid arthritis (RA).

Methods. Twenty patients with active RA were treated with 20 mg/kg of anti-TNFα in an open phase I/II trial lasting 8 weeks.

Results. The treatment was well tolerated, with no serious adverse events. Significant improvements were seen in the Ritchie Articular Index, which fell from a median of 28 at study entry to a median of 6 by week 6 ($P < 0.001$), the swollen joint count, which fell from 18 to 5 ($P < 0.001$) over the same period, and in the other major clinical assessments. Serum C-reactive protein levels fell from a median of 39.5 mg/liter at study entry to 8 mg/liter at week 6 ($P < 0.001$), and significant decreases were also seen in serum amyloid A and interleukin-6 levels.

Conclusion. Treatment with anti-TNFα was safe and well tolerated and resulted in significant clinical and laboratory improvements. These preliminary results support the hypothesis that TNFα is an important regulator in RA, and suggest that it may be a useful new therapeutic target in this disease.

Despite optimal use of current antirheumatic therapy, the outcome for many patients with rheumatoid arthritis (RA) consists of pain, disability, and premature death (1–3). As a response to the need for more effective and less toxic treatment, and to an increase in our understanding of the pathogenic mechanisms in RA, several groups have used monoclonal antibodies as therapeutic agents in this disease (4–10). Such immunotherapy has been, in most cases, targeted specifically to the T cell, a strategy based on evidence that T cells are involved in the initiation and maintenance of RA (11).

Here, we outline an alternative immunotherapeutic strategy, which involves the use of monoclonal antibodies with specificity for a cytokine, tumor necrosis factor α (TNFα). This approach is based on a body of knowledge regarding the role of cytokines in general, and of TNFα in particular, in the inflammatory process in RA. The first clearly documented study demonstrated the presence of interleukin-1 (IL-1) in RA synovial fluid (12). Subsequently, we and others have reported the presence and local synthesis in

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Table 1. Demographic features of 20 patients with refractory rheumatoid arthritis

Patient	Age/sex	Disease duration (years)	Previous DMARDs*	Concomitant therapy†
1	48/F	7	SSZ, DP, GST, AUR, MTX, AZA, HCQ	Pred. 5 mg
2	63/F	7	SSZ, GST, DP	Para. 1–2 gm
3	59/M	3	AUR, HCQ, GST, MTX, SSZ	Pred. 10 mg, Indo. 225 mg
4	56/M	10	GST, DP, AZA, SSZ	Pred. 12.5 mg, Ibu. 2 gm, Para. 1–2 gm
5	28/F	3	GST, SSZ, DP, AZA	Pred. 8 mg, Para. 1–2 gm, Code. 16 mg
6	40/M	3	SSZ, HCQ, AUR	Nap. 1 gm
7	54/F	7	DP, GST, SSZ, AZA, MTX	Para. 1–2 gm, Code. 16–32 mg
8	23/F	11	HCQ, GST, SSZ, MTX, AZA	Pred. 7.5 mg, Dicl. 100 mg, Para. 1–2 gm, Dext. 100–200 mg
9	51/F	15	GST, HCQ, DP, MTX	Pred. 7.5 mg, Dicl. 125 mg, Para. 1–3 gm
10	47/F	12	SSZ, CYC, MTX	Ben. 4 gm
11	34/F	10	DP, SSZ, MTX	Pred. 10 mg, Para. 1.5 gm, Code. 30–90 mg
12	57/F	12	GST, MTX, DP, AUR	Asp. 1.2 gm
13	51/F	7	SSZ, AZA	Para. 1–4 gm
14	72/M	11	GST, DP, AZA, MTX	Pred. 5 mg, Para. 1–4 gm, Code. 16–64 mg
15	51/F	17	HCQ, DP, SSZ, MTX	Asp. 0.3 gm
16	62/F	16	GST, DP, SSZ, MTX, AZA	Para. 1–4 gm, Code. 16–64 mg
17	56/F	11	SSZ, DP, GST, MTX, HCQ, AZA	Pred. 7.5 mg, Eto. 600 mg, Para. 1–2 gm, Dext. 100–200 mg
18	48/F	14	GST, MTX, DP, SSZ, AUR, AZA	Pred. 7.5 mg, Indo. 100 mg, Para. 1–3 gm
19	42/F	3	SSZ, MTX	Fen. 450 mg, Ben. 6 gm, Code. 30 mg
20	47/M	20	GST, DP, SSZ, AZA	Pred. 10 mg, Para. 1–3 gm

* Disease-modifying antirheumatic drugs (DMARDs) were SSZ = sulfasalazine; DP = D-penicillamine; GST = gold sodium thiomalate; AUR = auranofin; MTX = methotrexate; AZA = azathioprine; HCQ = hydroxychloroquine; CYC = cyclophosphamide.
† Daily doses are shown. Pred. = prednisolone; Para. = paracetamol; Indo. = indomethacin; Ibu. = ibuprofen; Code. = codeine phosphate; Nap. = naprosyn; Dicl. = diclofenac; Dext. = dextropropoxyphene; Ben. = benorylate; Asp. = aspirin; Eto. = etodolac; Fen. = fenbufen.

rheumatoid synovial membrane of many cytokines, including IL-1 (13), TNFα (13,14), IL-6 (15), granulocyte-macrophage colony-stimulating factor (GM-CSF; 16), IL-8 (17), and transforming growth factor β (TGFβ) (18,19).

We have investigated the relationships between these cytokines in RA, using a synovial culture system in which dissociated rheumatoid synovial cells are allowed to spontaneously re-aggregate in vivo. Even in the absence of extrinsic stimulation, such cells express high levels of cytokines and HLA class II molecules (20). Using this system, we showed that production of bioactive IL-1 was abrogated by neutralizing antibodies to TNFα, but not by antibodies to TNFβ or by normal rabbit IgG (21). This occurred in rheumatoid, but not osteoarthritic, cultures and suggested to us that TNFα was of particular importance as a regulatory cytokine. Subsequent analysis reinforced this concept, with the demonstration that another proinflammatory cytokine, GM-CSF, was regulated in the synovial membrane by TNFα (22) and that TNFα receptor expression, necessary for transmitting TNFα signals, was up-regulated in rheumatoid synovium (23,24).

Two recent mouse studies provide further insight into the importance of TNFα in arthritis. Keffer et al (25) described a mouse transgenic for the human TNFα gene, which expressed high levels of human TNFα in vivo and which reproducibly developed arthritis beginning at 4 weeks of age. The disease in these animals could be prevented by administration of monoclonal antibodies to human TNFα. In separate experiments in our own laboratory, we showed that in the type II collagen arthritis model in the DBA/1 mouse, the hamster anti-murine TNF monoclonal antibody TN3.19.2 significantly ameliorated the inflammation and tissue destruction when administered before or after the onset of disease (26).

Based on these considerations, it was of interest to determine the effect of therapy with a chimeric (human IgG1, murine Fv) monoclonal antibody to human TNFα in patients with rheumatoid arthritis. We report here that anti-TNFα therapy was safe and well tolerated, and induced marked improvements in both clinical and laboratory disease measures. These findings are consistent with our postulate concerning the critical role of TNFα in the pathogenesis of RA (27,28), and suggest that TNFα may be a useful therapeutic target in this disease.

Table 2. Changes in clinical assessments following treatment of rheumatoid arthritis patients with cA2*

Week of trial	Morning stiffness, minutes	Pain score, 0–10 cm	Ritchie index, 0–69	Swollen joint count, 0–28	Grip strength, 0–300 mm Hg		IDA, 1–4	Patient's assessment, no. grades improved, 0–3
					Left hand	Right hand		
Screen	135, 0–600	7.4, 4–9.7	23, 4–51	16, 4–28	84, 45–300	96, 57–300	3, 2.3–3.3	NA
0	180, 20–600	7.1, 2.7–9.7	28, 4–52	18, 3–27	77, 52–295	92, 50–293	3, 2–3.5	NA
1	20, 0–180 (<0.001†)	2.6, 0.6–7.8 (<0.001†)	13, 2–28 (<0.001; <0.002†)	13.5, 1–25 (>0.05)	122, 66–300 (>0.05)	133, 57–300 (>0.05)	2, 1.5–3.3 (<0.001†)	1, 1–3
2	15, 0–150 (<0.001†)	3.0, 0.3–6.4 (<0.001†)	13, 1–28 (<0.001†)	11.5, 1–22 (<0.003; <0.02†)	139, 75–300 (<0.03; >0.05†)	143, 59–300 (>0.05)	2, 1.5–3.2 (<0.001†)	1.5, 1–3
3	5, 0–150 (<0.001†)	2.2, 0.2–7.4 (<0.001†)	8, 0–22 (<0.001†)	6, 1–19 (<0.001; <0.002†)	113, 51–300 (>0.05)	142, 65–300 (>0.05)	2, 1.2–3.2 (<0.001†)	2, 1–2
4	15, 0–90 (<0.001†)	1.9, 0.1–5.6 (<0.001†)	10, 0–17 (<0.001†)	6, 0–21 (<0.001; <0.002†)	124, 79–300 (<0.02; >0.05†)	148, 64–300 (<0.03; >0.05†)	1.8, 1.3–2.7 (<0.001†)	2, 1–2
6	5, 0–90 (<0.001†)	1.9, 0.1–6.2 (<0.001†)	6, 0–18 (<0.001†)	5, 1–14 (<0.001†)	119, 68–300 (<0.04; >0.05†)	153, 62–300 (<0.05; >0.05†)	1.7, 1.3–2.8 (<0.001†)	2, 1–2
8	15, 0–60 (<0.001†)	2.1, 0.2–7.7 (<0.001†)	8, 1–28 (<0.001†)	7, 1–18 (<0.001†)	117, 69–300 (<0.03; >0.05†)	167, 53–300 (<0.03; >0.05†)	1.8, 1.5–2.8 (<0.001†)	2, 1–3

* Values are the median, range (*P*) for 20 patients for the initial screen and weeks 0–2, and for 19 patients thereafter. Patient 15 dropped out after week 2 of study. All *P* values versus week 0, by Mann-Whitney test. IDA = Index of Disease Activity; NA = not applicable.
† Adjusted for multiple statistical comparisons.

PATIENTS AND METHODS

Patient selection. Twenty patients were recruited, each of whom fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for the diagnosis of RA (29). The clinical characteristics of the patients are shown in Table 1. The study group comprised 15 females and 5 males, with a median age of 51 years (range 23–72), a median disease duration of 10.5 years (range 3–20), and a history of failed therapy with standard disease-modifying antirheumatic drugs (DMARDs) (median number of failed DMARDs 4, range 2–7).

Seventeen patients were seropositive at study entry or had been seropositive at some stage of their disease. All had erosions evident on radiographs of the hands or feet, and 3 had rheumatoid nodules. All patients had active disease at trial entry, as defined by an Index of Disease Activity (IDA) (30) of at least 1.75, together with at least 3 swollen joints, and were classified in anatomic and functional stage II or III (31). The pooled data for each of the clinical and laboratory indices of disease activity at the time of screening for the trial (up to 4 weeks prior to trial entry), and on the day of trial entry itself (week 0), are shown in Tables 2 and 3.

All DMARDs were discontinued at least 1 month prior to trial entry. Patients were allowed to continue taking a nonsteroidal antiinflammatory drug and/or prednisolone (≤12.5 mg/day) during the trial. The dosage of these agents was kept stable for 1 month prior to trial entry and during the course of the trial. No parenteral corticosteroids were allowed during these periods. Simple analgesics were allowed ad libitum.

Patients with other serious medical conditions were excluded from study. Specific exclusions were as follows: serum creatinine >150 μmoles/liter (normal 60–120), hemoglobin (Hgb) <90 gm/liter (normal 120–160 in females, and 135–175 in males), white blood cell (WBC) count <4 × 10^9/liter (normal 4–11 × 10^9/liter), platelet count <100 × 10^9/liter (normal 150–400 × 10^9/liter), and abnormal liver enzyme levels or active pathology noted on chest radiographs.

All patients gave their informed consent for the trial, and approval was granted by the local ethics committee.

Treatment protocol. cA2 is a chimeric human/mouse monoclonal anti-TNFα antibody, consisting of the constant regions of human (Hu)IgG1κ, coupled to the Fv region of a high-affinity neutralizing murine anti-HuTNFα antibody (A2). The antibody was produced by Centocor Inc., by continuous fermentation of a mouse myeloma cell line which had been transfected with cloned DNA coding for cA2, and was purified from culture supernatant by a series of steps involving column chromatography. The chimeric antibody retains specificity for natural and recombinant HuTNFα, and is of high affinity.

The antibody was stored at 4°C in 20-ml vials containing 5 mg of cA2 per milliliter of 0.01*M* phosphate buffered saline in 0.15*M* sodium chloride at a pH of 7.2 and was filtered through a 0.2-μm sterile filter before use. The appropriate amount of cA2 was then diluted to a total volume of 300 ml in sterile saline and administered intravenously via a 0.2-μm in-line filter over a period of 2 hours.

Patients were admitted to the hospital for 8–24 hours for each treatment, and were mobile except during infusions. The trial was of an open, uncontrolled design, with a comparison of 2 treatment schedules. Patients 1–5 and 11–20 received a total of 2 infusions, each consisting of 10 mg/kg of cA2, at entry to the study (week 0) and 14 days later (week 2). Patients 6–10 received a total of 4 infusions of 5 mg/kg at cA2, at entry and on days 4, 8, and 12. The total dose received by the 2 patient groups was therefore the same: 20 mg/kg.

Assessments. *Safety monitoring.* Vital signs were recorded every 15–30 minutes during infusions, and at intervals for up to 24 hours postinfusion. Patients were

Table 3. Changes in laboratory measures following treatment of rheumatoid arthritis patients with cA2*

Week of trial	Hgb, gm/liter	WBC, $\times 10^9$/liter	Platelets, $\times 10^9$/liter	ESR, mm/hour	CRP, mg/liter	SAA, mg/ml	RF, inverse titer
Screen	117, 98–146	7.9, 3.9–15.2	352, 274–631	59, 18–87	42, 9–107	ND	ND
0	113, 97–144	9.0, 4.9–15.7	341, 228–710	55, 15–94	39.5, 5–107	245, 18–1,900	2,560, 160–10,240
1	114, 96–145 (>0.05)	8.5, 3.6–13.6 (>0.05)	351, 223–589 (>0.05)	26, 13–100 (>0.05)	5, 0–50 (<0.001†)	58, 0–330 (<0.001; <0.003†)	ND
2	112, 95–144 (>0.05)	8.2, 4.3–12.7 (>0.05)	296, 158–535 (<0.04; >0.05†)	27, 10–90 (<0.02; >0.05†)	5.5, 0–80 (<0.001; <0.003†)	80, 11–900 (<0.02; <0.04†)	ND
3	110, 89–151 (>0.05)	9.0, 3.7–14.4 (>0.05)	289, 190–546 (<0.03; >0.05†)	27, 12–86 (<0.04; >0.05†)	7, 0–78 (<0.001; <0.002†)	ND	ND
4	112, 91–148 (>0.05)	8.2, 4.7–13.9 (>0.05)	314, 186–565 (>0.05)	23, 10–87 (<0.04; >0.05†)	10, 0–91 (<0.004; <0.02†)	ND	ND
6	116, 91–159 (>0.05)	9.1, 2.9–13.9 (>0.05)	339, 207–589 (>0.05)	23, 12–78 (<0.03; >0.05†)	8, 0–59 (<0.001†)	ND	ND
8	114, 94–153 (>0.05)	7.6, 4.2–13.5 (>0.05)	339, 210–591 (>0.05)	30, 7–73 (>0.05)	6, 0–65 (<0.001†)	ND	480, 40–5,120 (>0.05)

* Values are the median, range (*P*) for 20 patients for the initial screen and weeks 0–2, and for 19 patients thereafter. Patient 15 dropped out after week 2 of study. For rheumatoid factor (RF), only those patients with week 0 titers ≥1:160 in the particle agglutination assay were included (n = 14). All *P* values versus week 0, by Mann-Whitney test. Normal ranges: hemoglobin (Hgb) 120–160 gm/liter in females and 135–175 gm/liter in males; white blood cell (WBC) count 4–11 × 10^9/liter; platelet count 150–400 × 10^9/liter; erythrocyte sedimentation rate (ESR) <15 mm/hour in females and <10 mm/hour in males; C-reactive protein (CRP) <10 mg/liter; serum amyloid A (SAA) <10 mg/ml. ND = not done.

questioned concerning possible adverse events before each infusion and at weeks 1, 2, 3, 4, 6, and 8 of the trial. A complete physical examination was performed at screening and at week 8. In addition, patients were monitored by standard laboratory tests including a complete blood cell count, and levels of C3 and C4 components of complement, IgG, IgM, and IgA, serum electrolytes, creatinine, urea, alkaline phosphatase, aspartate transaminase, and total bilirubin.

Sample times for these tests were between 0800 and 0900 hours (preinfusion) and 1200–1400 hours (24 hours postinfusion). Blood tests subsequent to day 1 were performed in the morning, usually between 0700 and 1200 hours. Urine analysis and culture were also performed at each assessment point.

Response assessment. The patients were assessed for response to cA2 at weeks 1, 2, 3, 4, 6, and 8 of the trial. The assessments were all made between 0700 and 1300 hours by the same observer (AL-F). The following clinical assessments were made: duration of morning stiffness (minutes), pain score (0–10 cm on a visual analog scale), Ritchie Articular Index (maximum score 69) (32), number of swollen joints (28 joint count) (validation described in ref. 33), grip strength (0–300 mm Hg, mean of 3 measurements per hand, by sphygmomanometer cuff), and an assessment of function (the Stanford Health Assessment Questionnaire [HAQ], modified for British patients [34]). In addition, the patients' global assessments of response were recorded using a 5-point scale (worse, no response, fair response, good response, excellent response).

Routine laboratory indicators of disease activity included complete blood cell counts, C-reactive protein (CRP) levels (by rate nephelometry), and the erythrocyte sedimentation rate (ESR; Westergren). Followup assessments were made at monthly intervals after the conclusion of the formal trial period, in order to assess the duration of response.

Analysis of improvement in individual patients was made using two separate indices. First, an IDA was calculated for each time point according to the method of Mallya and Mace (30), with input variables of morning stiffness, pain score, Ritchie Articular Index, grip strength, ESR, and Hgb. The second index calculated was that of Paulus et al (35), which uses input variables of morning stiffness, ESR, joint pain/tenderness, joint swelling, and patient's and physician's global assessments of disease severity.

To calculate the presence (or otherwise) of a response according to this index, two approximations were made to accommodate our data. The swollen joint count used by us (nongraded total of swollen joints of 28 joints assessed), which has been validated (33), was used in place of the more extensive graded count described by Paulus et al, and the patient's and physician's global assessments of *response* recorded by us were approximated to the global assessments of *disease activity* used by Paulus et al (35). In addition to determining response according to these published indices, we selected 6 disease activity assessments of interest (morning stiffness, pain score, Ritchie Articular Index, swollen joint count, ESR, and CRP) and calculated their mean percentage improvement. We have used this value to give an indication of the degree of improvement seen in responding patients.

Immunologic investigations. Rheumatoid factors were measured using the rheumatoid arthritis particle agglutination assay (RAPA) (FujiBerio Inc, Tokyo, Japan), in which titers of 1:160 or greater were considered significant. Rheumatoid factor isotypes were measured by enzyme-linked immunosorbent assay (ELISA) (Cambridge Life Sciences, Ely, UK). Addition of cA2, at concentrations of up to 200 μg/ml, to these assay systems did not alter the assay results (data not shown).

Antinuclear antibodies were detected by immunoflu-

orescence on HEp-2 cells (Biodiagnostics, Upton, UK), and antibodies to extractable nuclear antigens were measured by counterimmunoelectrophoresis with polyantigen extract (Biodiagnostics). Sera positive by immunofluorescence were also screened for antibodies to DNA by the Farr assay (Kodak Diagnostics, Amersham, UK). Anticardiolipin antibodies were measured by ELISA (Shield Diagnostics, Dundee, Scotland). Serum amyloid A (SAA) was measured by sandwich ELISA (Biosource International, Camarillo, CA). Antiglobulin responses to the infused chimeric antibody were measured by an in-house ELISA, using cA2 as a capture reagent.

Cytokine assays. Bioactive TNF was measured in sera using the WEHI 164 clone 13 cytotoxicity assay (36). Total IL-6 was measured in sera using a commercial immunoassay (Medgenix Diagnostics, Brussels, Belgium) and using a sandwich ELISA developed in-house, with monoclonal antibodies provided by Dr. F. di Padova (Basel, Switzerland). Microtiter plates were coated with monoclonal antibody LNI 314-14 at a concentration of 3 μg/ml for 18 hours at 4°C, and blocked with 3% bovine serum albumin in 0.1*M* phosphate buffered saline, pH 7.2. Undiluted sera or standards (recombinant HuIL-6, 0–8.1 μg/ml) were added to the wells in duplicate and incubated for 18 hours at 4°C. Bound IL-6 was detected by incubation with monoclonal antibody LNI 110-14 for 90 minutes at 37°C, followed by biotin-labeled goat anti-murine IgG2b for 90 minutes at 37°C (Southern Biotechnology, Birmingham, AL). The assay was developed using streptavidin–alkaline phosphatase (Southern Biotechnology) and *p*-nitrophenyl phosphate as a substrate, and the optical density read at 405 nm.

Statistical analysis. Data for week 0 versus subsequent time points were compared for each assessment using the Mann-Whitney test. For comparison of rheumatoid factor titers (by RAPA), the data were expressed as dilutions before applying the test.

This was an exploratory study, in which prejudgments about the optimal times for assessment were not possible. Although it has not been common practice to adjust for multiple statistical comparisons in such studies (4–10), a conservative statistical approach would require adjustment of *P* values to take into account analysis at several time points. The *P* values have therefore been presented in two forms: unadjusted, and after making allowance for analysis at multiple time points by use of the Bonferroni adjustment. Where *P* values remained <0.001 after adjustment, a single value only is given. A *P* value of <0.05 is considered significant.

RESULTS

Safety of cA2. The administration of cA2 was exceptionally well tolerated, with no headache, fever, hemodynamic disturbance, allergy, or other acute manifestation. No serious adverse events were recorded during the 8-week trial. Two minor infective episodes were recorded, each "possibly related" to cA2: patient 15 presented at week 2 with clinical features of bronchitis. Sputum culture grew only normal commensals. She had a history of smoking and of a similar illness 3 years previously. The illness responded promptly to treatment with amoxicillin, but her second cA2 infusion was withheld and the data for this patient are therefore not analyzed beyond week 2. Patient 18 showed significant bacteriuria on routine culture at week 6 (>10^5/ml; lactose-fermenting coliform), but was asymptomatic. This condition also responded promptly to amoxicillin.

Routine analysis of blood samples showed no consistent adverse changes in hematologic parameters, renal function, liver function, or levels of C3, C4, or immunoglobulins during the 8 weeks of the trial. Four minor, isolated, and potentially adverse laboratory disturbances were recorded. Patient 2 experienced a transient rise in blood urea levels, from 5.7 mmoles/liter to 9.2 mmoles/liter (normal 2.5–7), with no change in serum creatinine. This change was associated with the temporary use of a diuretic, which had been prescribed for a non-rheumatologic disorder. The value normalized within 1 week and was classified as "probably not related" to cA2.

Patient 6 experienced a transient fall in the peripheral blood lymphocyte count, from 1.6 × 10^9/liter to 0.8 × 10^9/liter (normal 1.0–4.8). This abnormality was not seen at the next sample point (2 weeks later), was not associated with any clinical manifestations, and was classified as "possibly related" to cA2. Patients 10 and 18 developed elevated titers of anti-DNA antibodies at weeks 6 and 8 of the trial. Elevated anticardiolipin antibodies were also detected in patient 10. Both patients had a preexisting positive antinuclear antibody titer, and patient 10 had a history of borderline lymphocytopenia and high serum IgM. There were no clinical features of systemic lupus erythematosus, and the laboratory changes were judged "probably related" to cA2.

Efficacy of cA2. The pattern of response for each of the clinical assessments of disease activity and the derived IDA are shown in Table 2. All clinical assessments showed improvement following treatment with cA2, with maximal responses from week 3. Duration of morning stiffness decreased from a median of 180 minutes at study entry (week 0) to 5 minutes at week 6 ($P < 0.001$ by Mann-Whitney test, adjusted), representing a 97% improvement. The pain score decreased from 7.1 to 1.9 over the same period ($P < 0.001$, adjusted), representing an improvement of 73%. Similarly, the Ritchie Articular Index improved from 28 to 6 at week 6 ($P < 0.001$, adjusted; 79% improvement), and the swollen joint count decreased from 18

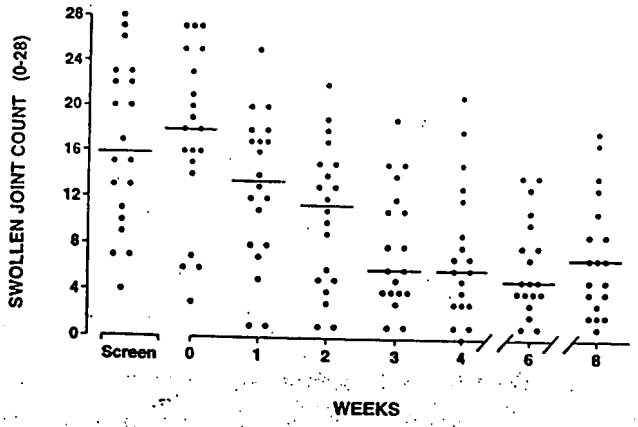

Figure 1. Swollen joint counts (maximum 28), as recorded by a single observer, in 20 patients with rheumatoid arthritis treated with cA2. The screening time point was within 4 weeks of entry to the study (week 0); data from patient 15 were not included after week 2 (dropout). Significance of the changes, relative to week 0, were determined by Mann-Whitney test (adjusted): $P > 0.05$ at week 1, $P < 0.02$ at week 2, $P < 0.002$ at weeks 3 and 4, and $P < 0.001$ at weeks 6 and 8. Bars show median values.

to 5 ($P < 0.001$, adjusted; 72% improvement). The individual swollen joint counts for all time points are shown in Figure 1.

Grip strength also improved; the median grip strength rose from 77 mm Hg (left) and 92 mm Hg (right) at week 0 to 119 (left) and 153 (right) at week 6 ($P < 0.04$ and $P < 0.05$, left and right hands, respectively; $P > 0.05$ both hands, adjusted for multiple comparisons). The IDA has a range of 1 (normal) to 4 (severe disease activity). The IDA showed a decrease from a median of 3 at study entry to 1.7 at week 6 ($P < 0.001$, adjusted). Patients were asked to grade their responses to cA2 using a 5-point scale. No patient recorded a response of "worse" or "no change" at any point in the trial. "Fair," "good," and "excellent" responses were classified as improvements of 1, 2, and 3 grades, respectively. At week 6, there was a median of 2 grades of improvement (Table 2).

We also measured changes in the patients' functional capacity, using the HAQ, as modified for British patients (range 0–3). The median (range) HAQ score improved from 2 (0.9–3) at study entry to 1.1 (0–2.6) by week 6 ($P < 0.001$ and $P < 0.002$ adjusted).

The changes in the laboratory values which reflect disease activity are shown in Table 3. The most rapid and impressive changes were seen in serum CRP levels, which fell from a median of 39.5 mg/liter at week 0 (normal <10) to 8 mg/liter by week 6 of the trial ($P < 0.001$, adjusted), representing an improvement of 80%. Of the 19 patients with elevated CRP at study entry, 17 showed decreases to the normal range at some point during the trial. The improvement in CRP was maintained in most patients over the assessment period (Table 3 and Figure 2); the exceptions with high values at 4 and 6 weeks tended to be those with the highest starting values (data not shown).

The ESR also showed improvement, with a fall from 55 mm/hour at study entry (normal <10 in males and <15 in females) to 23 mm/hour at week 6 ($P < 0.03$ and $P > 0.05$ adjusted; 58% improvement). SAA levels were elevated in all patients at trial entry, and fell from a median of 245 mg/ml (normal <10) to 58 mg/ml at week 1 ($P < 0.003$ adjusted; 76% improvement) and to 80 mg/ml at week 2 ($P < 0.04$, adjusted). No significant changes were seen in Hgb level, WBC count, or platelet count at week 6, although the platelet count did improve at weeks 2 and 3 compared with trial entry (Table 3).

The response data were also analyzed for each patient individually (not shown). The majority of patients had their best overall responses at week 6, at which time 13 assessed their responses as "good" while 6 assessed their responses as "fair." Eighteen of the 19 patients who completed the treatment schedule achieved an improvement in the IDA of 0.5 or greater at week 6, and 10 achieved an improvement of 1.0 or greater. All patients achieved a response at week 6

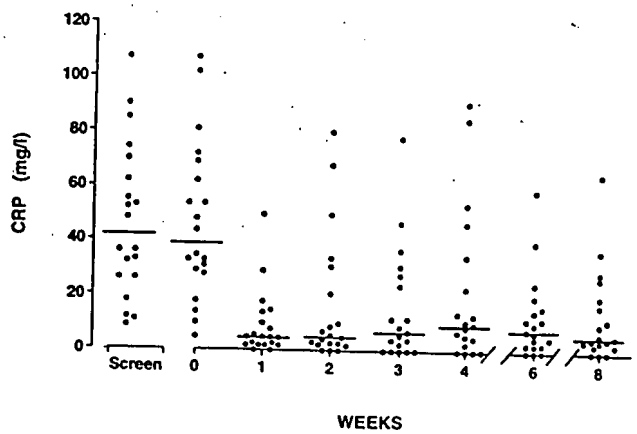

Figure 2. Serum C-reactive protein (CRP) levels (normal 0–10 mg/liter), as measured by nephelometry, in 20 patients with rheumatoid arthritis treated with cA2. The screening time point was within 4 weeks of entry to the study (week 0); data from patient 15 were not included after week 2 (dropout). Significance of the changes, relative to week 0, were determined by Mann-Whitney test (adjusted): $P < 0.001$ at week 1, $P < 0.003$ at week 2, $P < 0.002$ at week 3, $P < 0.02$ at week 4, and $P < 0.001$ at weeks 6 and 8. Bars show median values.

according to the index described by Paulus et al (35). At week 6, all patients showed a mean improvement of 30% or greater in the 6 selected measures of disease activity (see Patients and Methods), with 18 of the 19 patients showing a mean improvement of 50% or greater (data not shown).

Although the study was primarily designed to assess the short-term effects of cA2 treatment, followup clinical and laboratory data are available for those patients followed for sufficient time (n = 12). The duration of response in these patients, defined as the duration of a 30% (or greater) mean improvement in the 6 selected disease activity measures, was variable, ranging from 8 weeks to 25 weeks (median 14) (data not shown).

Comparison of the clinical and laboratory data for patients treated with 2 infusions of cA2 (each at 10 mg/kg) versus those treated with 4 infusions (each at 5 mg/kg) showed no significant differences in the rapidity or extent of response (data not shown).

Immunologic investigations and cytokines. Measurement of rheumatoid factor by RAPA showed 14 patients with significant titers (≥1:160) at trial entry. Of these, 6 patients showed a decrease of at least 2 titers on treatment with cA2, while the remaining patients showed a change of 1 titer or less. No patient showed a significant increase in rheumatoid factor titer during the trial (data not shown). The median titer in the 11 patients decreased from 1:2,560 at entry to 1:480 by week 8 ($P > 0.05$) (Table 3). Specific rheumatoid factor isotypes were measured by ELISA, and showed decreases in the 6 patients whose RAPA had declined significantly, as well as in some other patients (data not shown). Median values for the 3 isotypes in the 14 patients seropositive at trial entry were 119, 102, and 62 IU/ml (IgM, IgG, and IgA isotypes, respectively) and at week 8 were 81, 64, and 46 IU/ml ($P > 0.05$).

We tested sera from patients 1–9 for the presence of bioactive TNF, using the WEHI 164 clone 13 cytotoxicity assay (36). In 8 patients, serum samples spanning the entire trial period were tested, while for patient 9, only 3 samples (1 pretrial, 1 intermediate, and the last available sample) were tested. The levels of bioactive TNF were below the limit of sensitivity of the assay in the presence of human serum (1 pg/ml) (data not shown).

Since production of CRP and SAA are thought to be regulated in large part by IL-6, we also measured serum levels of this cytokine, using 2 different assays which measure total IL-6. In the Medgenix assay, IL-6 was significantly elevated in 17 of the 20 patients at study entry. In this group, levels fell from 60 pg/ml (range 18–500) to 40 pg/ml (range 0–230) at week 1 ($P > 0.05$) and to 32 pg/ml (range 0–210) at week 2 ($P < 0.005$ and $P < 0.01$, adjusted). These results were supported by measurement of serum IL-6 in the first 16 patients in a separate ELISA developed in-house. IL-6 was detectable in 11 of these samples, with median (range) levels falling from 210 pg/ml (25–900) at entry to 32 pg/ml (0–1,700) at week 1 ($P < 0.02$ and $P < 0.04$, adjusted) and to 44 pg/ml (0–240) at week 2 ($P < 0.02$ and $P < 0.03$, adjusted).

We tested sera from patients 1–10 for the presence of antiglobulin responses to the infused chimeric antibody, but none were detected (data not shown). In many patients, however, cA2 was still detectable in serum samples taken at week 8 (data not shown) and this may have interfered with the ELISA.

DISCUSSION

This is the first report describing the administration of anti-TNFα antibodies for treatment of human autoimmune disease. Many cytokines are produced in rheumatoid synovium, but we chose to specifically target TNFα because of mounting evidence that it was a major molecular regulator in RA (21,22,26–28). The study results presented here support that view and allow 3 important conclusions to be drawn.

First, treatment with cA2 was safe and the infusion procedure was well tolerated. Although fever, headache, chills, and hemodynamic disturbance have all been reported following treatment with anti-CD4 or anti-CDw52 in RA (6,10), such features were absent in our patients. Also notable was the absence of any allergic event despite repeated treatment with the chimeric antibody, although the interval between initial and repeat infusions may have been too short to allow maximal expression of any antiglobulin response. The continuing presence of circulating cA2 at the conclusion of the trial may have precluded detection of antiglobulin responses, but also indicated that any such responses were likely to be of low titer and/or affinity. Although we recorded 2 episodes of infection among the study group, these were minor and the clinical courses were unremarkable. TNFα has been implicated in the control of *Listeria* and other infections in mice (37), but our limited experience does not suggest an increased risk of infection after TNFα blockade in humans.

The second conclusion concerns the clinical

efficacy of cA2. The patients we treated had longstanding, erosive, and for the most part, seropositive disease, and therapy with several standard DMARDs had failed. Despite this, the major clinical assessments of disease activity and outcome (morning stiffness, pain score, Ritchie articular index, swollen joint count, and HAQ score) showed statistically significant improvement, even after adjustment for multiple comparisons. All patients graded their response as at least "fair," with the majority grading it as "good." In addition, all achieved a response according to the criteria of Paulus et al and showed a mean improvement of at least 30% in 6 selected disease activity measures. The design of the trial does not allow these results to be attributed to the action of cA2 alone. However, the extent of the clinical improvements, their consistency throughout the study group, and the parallel changes in laboratory indices of disease activity (see below) are encouraging.

The improvements in clinical assessments following treatment with cA2 appear to be at least as good as those reported following treatment of similar patients with antileukocyte antibodies (6,10), although firm conclusions concerning each of these agents will require controlled, blinded studies. The two therapeutic approaches may already be distinguished, however, by their effects on the acute-phase response, since in several studies of antileukocyte antibodies, no consistent improvements in CRP or ESR were seen (4–6,8,10). In contrast, treatment with cA2 resulted in significant decreases in serum CRP and SAA values, with normalization of values in many patients. The changes were rapid and marked, and in the case of CRP, sustained for the duration of the study (Table 3). The decreases in ESR were less marked, achieving statistical significance only when unadjusted for the number of comparisons (Table 3).

These results are consistent with current concepts that implicate TNFα in the regulation of hepatic acute-phase protein synthesis, either directly, or by control of other, secondary, cytokines such as IL-6 (38,39). To investigate the mechanism of control of the acute-phase response in our patients, we measured serum TNFα and IL-6 before and after cA2 treatment. Bioactive TNFα was not detectable in sera obtained at baseline or subsequently. In view of previous reports of variability between different immunoassays in the measurement of cytokines in biologic fluids (40), we used 2 different assays for IL-6, and both demonstrated significant decreases in serum IL-6 levels by week 2. These findings support the other objective laboratory changes induced by cA2, and provide in vivo evidence that TNFα may be a regulatory cytokine for IL-6 in this disease. Among the other laboratory tests performed, levels of rheumatoid factors fell significantly in 6 patients.

The mechanism of action of cA2 leading to the clinical responses outlined above was not established in this study. Neutralization of TNFα may have a number of beneficial consequences, including a reduction in the local release of cytokines such as IL-6 and other inflammatory mediators, and modulation of synovial endothelial/leukocyte interactions. cA2 may also bind directly to synovial inflammatory cells expressing membrane TNFα, with subsequent in situ cell lysis. Further studies should establish which actions of cA2 may be clinically important.

The results obtained in this small series have important implications, both scientifically and clinically. At the scientific level, the ability of the neutralizing antibody, cA2, to reduce acute-phase protein synthesis, reduce the production of other cytokines such as IL-6, and significantly improve the clinical state demonstrates that it is possible to interfere with the cytokine network in a useful manner without untoward effects. Due to the many functions and overlapping effects of cytokines such as IL-1 and TNFα, and the fact that cytokines induce the production of other cytokines and of themselves, there had been some pessimism as to whether targeting a single cytokine in vivo would have any beneficial effect (41,42). This view is clearly refuted. On the clinical side, the results of short-term treatment with cA2 are encouraging, and suggest that TNFα may be a useful new therapeutic target in RA.

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